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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/017,372	10/19/2001	Lawrence A. Wolfraim	4239-61302	6866

7590 06/17/2003

KLARQUIST SPARKMAN, LLP
One World Trade Center, Suite 1600
121 SW Salmon Street
Portland, OR 97204

EXAMINER
NICHOLS, CHRISTOPHER J

ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 06/17/2003

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	10/017,372	WOLFRAIM ET AL.	
	Examiner	Art Unit	
	Christopher Nichols, Ph.D.	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *28 March 2003* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-37,49-53 and 55* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-37,49-53 and 55* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *18 June 2002* is/are: a)☐ accepted or b)☒ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *9* .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

Election/Restrictions

1. Applicant's election with traverse of Group I (claims 1-37 and 49-56), Group Y (SEQ ID NO: 36), and Group I (tags) in Paper No. 11 (14 January 2003) is acknowledged. The traversal is on the ground(s) that all Groups should be examined together. This is not found persuasive because each invention in Groups I-IV represents a separate and distinct invention. Examination of all four groups presents an undue burden on the Examiner. In terms of the second restriction requirement between Groups A-H, U-Z, AA, BB, and CC each group represents a distinct and independent sequence that requires its own individual and non-coextensive search thus presenting a search burden on the Examiner. Concerning the third restriction requirement, as set forth at ¶11-14 pp. 8-9, Groups i-iv, the Applicant's argument is found persuasive and is hereby *withdrawn*. The Applicant further requests that Group Z (SEQ ID NO: 37) to be rejoined with Group Y (SEQ ID NO: 36) because the polynucleotide SEQ ID NO: 37 encodes the polypeptide SEQ ID NO: 36. The Applicant's request is granted. Group Z and Group Y are hereby rejoined and will be examined with Group I (claims 1-37 and 49-56) drawn to a TGF-β family fusion protein, isolated nucleic acid molecules, vectors, and host cells comprising same (including SEQ ID NO: 36 and SEQ ID NO: 37). The remaining restriction requirements are still deemed proper and are therefore made FINAL.

Status of Application, Amendments, and/or Claims

2. The Preliminary Amendments filed 18 June 2002 (Paper No. 6), 14 January 2003 (Paper No. 11), and 28 March 2003 (Paper No. 13) have been received and entered in full. Claims 38-

48, 54, and 56 have been cancelled. Claims 11, 31, 33, and 49 have been amended. Claims 1-37, 49-53, and 55 are under examination.

Drawings

3. The drawings are objected to because the labels on Figure 4B are overrun and unclear. A proposed drawing correction or corrected drawings are required in reply to the Office action to avoid abandonment of the application. The objection to the drawings will not be held in abeyance.

Specification

4. The disclosure is objected to because of the following informalities: misspelling "phsopho" (pp. 4 line 25). Appropriate correction is required.

Claim Objections

5. Claims **1-37, 49-53,** and **55** are objected to because of the following informalities: the claims recite non-elected material. Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

6. Claims **10, 11, 14-17, 20-24, 31, 33, 49-53**, and **55** are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for *SEQ ID NO: 36 and SEQ ID NO: 37 with a FLAG or HA tag, vectors, and host cell comprising same wherein SEQ ID NO: 36 encodes TGF-β_1, and SEQ ID NO: 37 is TGF-β_1*, does not reasonably provide enablement for *SEQ ID NO: 36 or SEQ ID NO: 37 with substations, insertions, fragments, sequence derivatives thereof, other members of the TGF-β family, or other molecular biology tags*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make the invention commensurate in scope with these claims.

7. The claims are drawn very broadly to SEQ ID NO: 36 and SEQ ID NO: 37 and derivatives, fragments, sequences with substitutions thereof, and numerous molecular biology tags. The language of said claims encompasses three types of mutation or variation, derivitization, where a sequence need only share 85% sequence homology with either SEQ ID NO: 36 or SEQ ID NO: 37, fragments, where only specific stretches of SEQ ID NO: 36 and SEQ ID NO: 37 are necessary to comprise the invention, and substitutions, which can lead to frameshift mutations.

8. The specification teaches that TGF-β family proteins are not tolerant of mutation and are especially sensitive to the insertion or inclusion of tags common in molecular biology, including but not limited to antigen-tags, enzymatic tags, and fluorescent tags. Do to this intolerance, those skilled in the art usually resorted to an I^{125} tag which is undesirable for reasons set forth in the specification. Thus a motivation and desire exists to use non-radioactive tags for TGF-β family proteins that preserve biological activity. The specification teaches that this was accomplished using a FLAG and a HA tag.

9. The specification as filed does not provide any guidance or examples that would enable a skilled artisan to use tags other than HA and FLAG successfully. Additionally, a person skilled in the art would recognize that predicting the efficacy of using a specific tag based solely on the performance of FLAG and HA (both known small epitope tags) is highly problematic. Thus, although the specification prophetically considers and discloses general methodologies of other larger tags such as enzymatic or fluorescent tags, such a disclosure would not be considered enabling since the state of recombinantly tagging TGFβ_1 is highly unpredictable. The factors listed below have been considered in the analysis of enablement:

(A) The breadth of the claims;
(B) The nature of the invention;
(C) The state of the prior art;
(D) The level of one of ordinary skill;
(E) The level of predictability in the art;
(F) The amount of direction provided by the inventor;
(G) The existence of working examples; and
(H) The quantity of experimentation needed to make or use the invention based on the content of the disclosure.

10. The following references are cited herein to illustrate the state of the art of TGF-β family proteins.

11. Concerning the breadth of the claims, Huang *et al.* (24 September 1999) "An Active Site of Transforming Growth Factor-β_1 for Growth Inhibition and Stimulation." The Journal of Biological Chemistry **274**(39): 27754-27758 (**IDS**) teaches that a single amino acid change can greatly diminish TGF-β_1 activity (Abstract; Figure 2). Thus it is an undue burden of experimentation exists for the skilled artisan to practice the claims to their full breath (including "substitutions", "fragments", "derivatives", and "mutations").

12. Concerning the nature of the invention, Han *et al.* (1997) "Refolding of a Recombinant Collagen-Targeted TGF-β_2 Fusion Protein Expressed in *Escherichia coli*." Protein Expression and Purification **11**: 169-178 (**IDS**) teaches that the recombinant purification of TGF-β family members is difficult (pp. 169). While Han *et al.* (1997) do succeed in tagging, expressing, and purifying a TGF- β_2 fusion protein; the yields were very low (pp. 170). Also, Han *et al.* (1997) notes that TGF-β isoforms while they share on average 65% sequence homology, greatly differ in their potency and physiological effects (pp. 169). Thus the skilled artisan is presented with the daunting task of determining, *de novo*, which tags are acceptable and then to characterize each sequence variant, fragment, and derivative to ensure it exhibits the desired biological activity.

13. On the use of tags and derivatives of TGF-β, Qian *et al.* (29 November 1996) "Binding Affinity of Transforming Growth Factor-β for Its Type II Receptor Is Determined by the C-Terminal Region of the Molecule." The Journal of Biological Chemistry **271**(48): 30656-30662 (**IDS**) teaches that single amino acid changes and small deletions (6 residues) will detrimentally affect TGF-β_1 binding properties (Figures 1-4). Also Wakefield *et al.* (1991) "Addition of a C-Terminal Extension Sequence to Transforming Growth Factor-β_1 Interferes with Biosynthetic Processing and Abolishes Biological Activity." Growth Factors **5**: 243-253 (**IDS**) teaches that the addition of a 6-residue epitope tag abolishes TGF-β_1 activity (Table 1).

14. In regards to the unpredictability of the effects of a tag on TGFβ1, Wolfraim *et al.* (1 August 2002) "Development and application of fully functional epitope-tagged forms of transforming growth factor-β." Journal of Immunological Methods **266**(1-2): 7-18 teaches that attempts to introduce a tag into TGFβ1 have encountered problems with the LAP domain, protein folding, low yield, and lack of biological activity (pp. 16). It is noted, however, that

Wolfraim et al. (2002) did succeed in tagging TGFβ_1 with HA and FLAG, hence the scope of enablement rejection.

15. Regarding derivatives and fragments of SEQ ID NO: 36 and SEQ ID NO: 37, the problem of predicting protein structure from sequence data and in turn utilizing predicted structural determinations to ascertain functional aspects of the protein is extremely complex. While it is known that many amino acid substitutions are generally possible in any given protein the positions within the protein's sequence where such amino acid substitutions can be made with a reasonable expectation of success are limited. Certain positions in the sequence are critical to the protein's structure/function relationship, e.g. such as various sites or regions directly involved in binding, activity and in providing the correct three-dimensional spatial orientation of binding and active sites. These or other regions may also be critical determinants of antigenicity. These regions can tolerate only relatively conservative substitutions or no substitutions (see Wells, 1990, Biochemistry 29:8509-8517; Ngo et al., 1994, The Protein Folding Problem and Tertiary Structure Prediction, pp. 492-495). However, Applicant has provided little or no guidance beyond the mere presentation of sequence data to enable one of ordinary skill in the art to determine, without undue experimentation, the positions in the protein which are tolerant to change (e.g. such as by amino acid substitutions or deletions), and the nature and extent of changes that can be made in these positions. Although the specification outlines art-recognized procedures for producing and screening for active muteins, this is not adequate guidance as to the nature of active derivatives that may be constructed, but is merely an invitation to the artisan to use the current invention as a starting point for further experimentation. Even if an active or binding site were identified in the specification, they may not be sufficient, as the ordinary

artisan would immediately recognize that an active or binding site must assume the proper three-dimensional configuration to be active, which conformation is dependent upon surrounding residues; therefore substitution of non-essential residues can often destroy activity. The art recognizes that function cannot be predicted from structure alone (Bork, 2000, Genome Research 10:398-400; Skolnick et al., 2000, Trends in Biotech. 18(1): 34-39, especially p. 36 at Box 2; Doerks et al., 1998, Trends in Genetics 14:248-250; Smith et al., 1997, Nature Biotechnology 15:1222-1223; Brenner, 1999, Trends in Genetics 15:132-133; Bork et al., 1996, Trends in Genetics 12:425-427). Due to the large quantity of experimentation necessary to generate the infinite number of derivatives recited in the claims and possibly screen same for activity, the lack of direction/guidance presented in the specification regarding which structural features are required in order to provide activity, the absence of working examples directed to same, the complex nature of the invention, the state of the prior art which establishes the unpredictability of the effects of mutation on protein structure and function, and the breadth of the claims which fail to recite any structural or functional limitations, undue experimentation would be required of the skilled artisan to make and/or use the claimed invention in its full scope.

16. Since the specification fails to provide any guidance for the successful use of other tags and since resolution of the various complications in regards to including a molecular biology tag on a TGF-β protein and preserving biological activity is highly unpredictable, one of skill in the art would have been unable to practice the invention without engaging in undue trial and error experimentation. In order to practice the invention using the specification and the state of the prior art as outlined above, the quantity of experimentation required to practice the invention as claimed would require the *de novo* determination of constructs and their testing. In the absence

of any guidance from the specification, the amount of experimentation would be undue, and one would have been unable to practice the invention over the scope claimed.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

17. Claims **1** is rejected under 35 U.S.C. 102(b) as being anticipated by Jakowlew *et al.* (1988) "Nucleotide sequence of chicken transforming growth factor-beta 1 (TGFβ 1)." Nucleic Acids Research **16**(17): 8730 teaches a sequence which has 88.3% homology to SEQ ID NO: 36 and 92.7% sequence homology to SEQ ID NO: 37 thus meeting the limitations of claim 1 (pp. 8730).

18. Claims **1, 25**, and **27-30** are rejected under 35 U.S.C. 102(b) as being anticipated by WO 96/39430 (12 December 1996) Hall *et al* (**IDS**). WO 96/39430 teaches a fusion protein of TGF-β, TGF-β_1, TGF- β_2, and TGF- β_3 that retains its biological activity thus meeting the limitations of claims 1 and 25 (pp. 3 lines 15-33). WO 96/39430 teaches a fusion protein of TGF-β, TGF-β_1, TGF- β_2, and TGF- β_3 that retains its biological activity wherein the fusion partner is a purification tag such as a His tag, a GST tag, or a HA tag thus meeting the limitations of claims 1, 25, and 27-30 (pp. 50, claims 1-7).

19. Claims **1-9, 12, 13, 18, 19, 25-30, 32**, and **34-37** are rejected under 35 U.S.C. 102(b) as being anticipated by US 5800811 (1 September 1998) Hall *et al.* US 5800811 teaches a transforming growth factor-β fusion protein including TGF-β_1, TGF-β_2, and TGF-β_3 thus meeting the limitations of claim 1 and 27 (Abstract). US 5800811 teaches a fusion protein of TGF-β_1, TGF-β_2, and TGF-β_3 and a tag wherein the resultant fusion protein is a mature, biologically active, dimmer thus meeting the limitations of claims 1, 2, 3, 5, and 28 (Col. 1 lines 45-67; Col. 2 lines 10-67; Example 7; Example 11). US 5800811 teaches that the tags included on the N-terminus of TGF-β_1 can be GST, HA, HIS_6, epitope tags, enzymes, and fragments of TGF-β_2, and TGF-β_3 this includes the "pro-region" thus meeting the limitations of claims 6, 7, 8, 9, 12, 13, 18, 19, 25, 27, 28, 29, and 30 (Col. 3 and Col. 4). US 5800811 also teaches that the fusion protein and expression vectors thereof including nucleic acids and thus sequences can be expressed in *E. coli* or other suitable hosts thus meeting the limitations of claims 4, 32, 34, 35, and 36 (Col. 4 lines 40-67; Example 1).

20. Claims **1-9, 12, 13, 18, 19, 32**, and **34-37** are rejected under 35 U.S.C. 102(b) as being anticipated by US 5221620 (22 June 1993) Purchio *et al.* US 5221620 teaches the expression and purification of TGF-β_2, a member of the transforming growth factor-β fusion protein as a biologically active dimmer in Chinese hamster ovary cells (CHO; a mammalian cell line) thus meeting the limitations of claim 1-9 (Col. 1 lines 1-45). US 5827733 teaches the expression of TGF-β_2 in prokaryotes and eukaryotes such as yeast and *E. coli* as a fusion protein with the pro-region of TGF-β_1 thus meeting the limitations of claims 12, 13, 18, 19, 32, and 34-37 (Col. 9 lines 20-64; Col. 10 lines 29-68; Col. 36 lines 20-56).

Summary

21. Claims **1-37, 49-53**, and **55** are hereby rejected.

22. The following articles and patents were found by the Examiner during the art search and are of note:

a. US 2003/0027218 A1 (06 February 2003) Yamasaki et al. (SEQ ID NO: 26 of '218 shares 94.0% sequence homology with SEQ ID NO: 37 of the instant application).

b. Brunner *et al.* (15 August 1989) "Site-directed Mutagenesis of Cysteine Residues in the Pro Region of the Transforming Growth Factor b1 Precursor." The Journal of Biological Chemistry **264**(23): 13660-13664.

c. US 5827733 (27 October 1998) Lee *et al.*

d. US 5304541 (19 April 1994) Purchio *et al.* (Discloses sequences that share 67.8% homology with SEQ ID NO: 36 and 88.7% sequence homology with SEQ ID NO: 37).

e. WO 01/81404 A2 (1 November 2001) Strober *et al.* (Discloses sequence that shares 89.0% sequence homology with SEQ ID NO: 36).

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Christopher James Nichols, Ph.D.** whose telephone number is 703-305-3955. The examiner can normally be reached on Monday through Friday, 8:00AM to 5:00PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Gary Kunz, Ph.D.** can be reached on 703-308-4623. The fax phone numbers for the organization where this application or proceeding is assigned are 703-872-9306 for regular communications and 703-872-9307 for After Final communications. The fax phone numbers for the customer service center is 703-872-9305

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is 703-308-0196.

Elizabeth C. Kemmerer

CJN
June 2, 2003

ELIZABETH KEMMERER
PRIMARY EXAMINER